

From Page No. 26

HPK6
(Full length) $= 0.382 \times 50 = 19.1 \text{ mo} \div 10 = 1.9$

$$\text{Fu2 29} \quad = 0.416 \times 50 = 20.8 \div 10 = 2.08 \frac{\mu\text{e}}{\mu}$$

Picked up DP12 (CHO) cells for transfection
on 5/14

Have 50ml
put into a spinne
flask

Added 100ml
F12 + 5% dFBS
+ 10mM HEPES
+ 1X GAT

Inc 37°C o/n.

Genentech, Inc. Genentech, Inc. Genentech, Inc. Genentech, Inc. Genentech, Inc.		INTERNAL TRANSMITTAL FORM	
TO: Will Buren		DEPARTMENT NAME AND CODE CENTER NO. 409	
FROM: C. Nagel		DEPARTMENT NAME AND CODE CENTER NO. 613	
SUBJECT: 708-215		EXPIRATION DATE: 50 ml	
STOCK NUMBER: 10594 -			
STORAGE CONDITIONS:			
SPECIAL INSTRUCTIONS:			
BSA + 5% NPBS + 1X GMT			
cont. 22.0 x 10 ⁵ Clnl (98%)			

To Page No. 4

Date _____

Recorded by _____

From Page No. 87

RD's for transfection

(A) full length

26.2 μ l DNA (50 μ g)
 10 μ l 10x H
 61.8 μ l H₂O
 2 μ l Not 1 (80.4)
 100

(B) Fusion

24 μ l DNA (50 μ g)
 10 μ l 10x H
 64 μ l H₂O
 2 μ l Not 1 (80.4)
 100

Inc both 37°C 3hrs

Saved 1 μ l each for gel analysis

Extracted each 1x 100 μ l 50/50 phenol/CHCl₃
 1x 100 μ l CHCl₃

Added 10 μ l 3M NaAc pH 4.8 to each
 Added 220 μ l RT 100% EtOH to each

Mixed + spun full speed, RT 5'

Decanted

Washed pellets 2x 1ml -20°C 70% EtOH

Respun \rightarrow decantedRes'd each in 100 μ l H₂O \rightarrow kept on iceRemoved 1 μ l each for gel analysis

Ran pre + post ppt samples on 0.7%
 agarose (1x TBE)

To Page No. 89

Witnessed & Understood by me,

Date

Invented by

Date

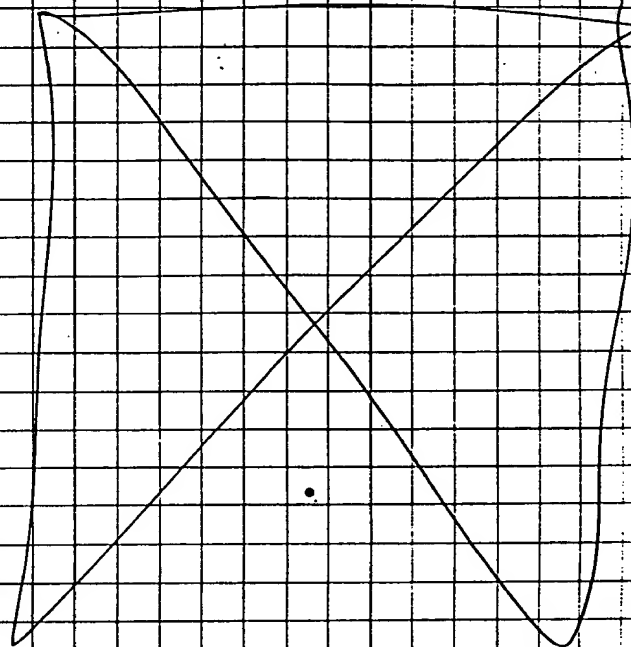
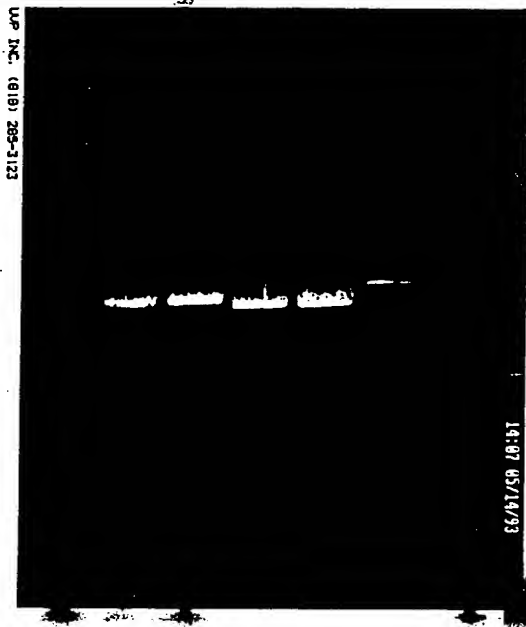
Recorded by

WMA Brown

TITLE

From Page No. 88

READY!



Counted 200ul of DP12 cells in coulter counter,
Have 7.6×10^5 cells/ml

need 1×10^7 cells / transfection = 13.2ml

Spun down 2x 13.2ml ~2K RPM 6PR 4°C 5'

Decanted & RS'd each in 700ul 2x Hapes buffered saline
Placed in pre-chilled Bio-Rad 0.4cm electroporation cuvette
Added DNA's to cells

Electroporated 25µF, 0.5 Kvoults

Got 0.4 ~~cells~~ \rightarrow removed from cuvette
ms (time constant)

To Page No. 91

Witnessed & Understood by me,

Date

Invented by

Recorded by

Date

W. H. Brown

[Signature]

90

From Page No. 89

Plated each transfection onto 4x100mm
tissue culture plates

w/ 10ml F12 5% dFBS
DMEM 10mM HEPES
1x SHT

Inc @ 37°C w/ 5% CO₂ o/n.

To Page No. 91

Witnessed & Understood by me,

Date

Invented by

Recorded by

W. M. Brown

Date

TITLE

From Page No. 90

Checked cells \rightarrow I don't have a proper microscope, but it appears that they mostly all stuck down.

Changed media (after 24 hr incubation) to DMEM + 5% FBS + 10 mM HEPES.

\ominus GHT (for selection of DHFR intro on pSV15)

Note: I may have accidentally used 100 mM HEPES on 5/18 after electroporation. I will have to wait & see if this causes a problem.

To Page No. 92

Witnessed & Understood by me,

Date

Invented by

Recorded by

Date

Project N 713
Book No. 17522 TITLE _____

92

From Page No. 91

Got a tissue culture scope today + checked
cells → good deal

Many dead ones in there, but still
plates are ~ 50% confluent.

Changed media

Continued Inc 37°C o/n 5% CO₂.

To Page No. 93

Witnessed & Understood by me,

Date

Invented by

Recorded by

Date

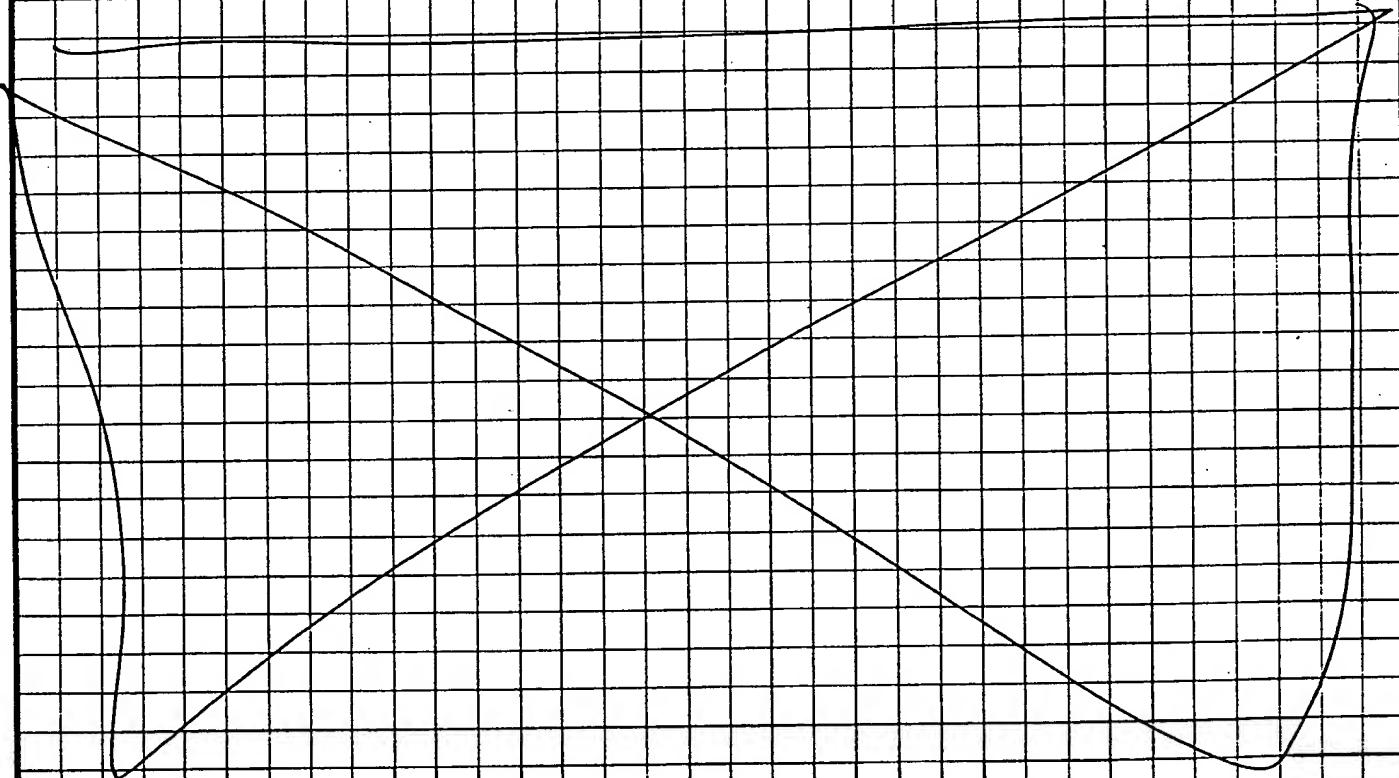
TITLE _____

From Page No. 92

Checked CHO cells today. I am starting to get very much more cell death (probably due to the GHT⁺ selection).

I should eventually have essentially clear plates except for the isolated colonies of transformed cells.

Continued incubation @ 37°C w/ 5% CO₂.



To Page No. 94

Witnessed & Understood by me, _____

Date _____

Invented by _____

Date _____

Recorded by _____

W. M. Baron

[Signature]

Project No. 1713
 Book No. 17522 TITLE _____

94

From Page No. 93

Checked CHO cell cultures

HPTK6 full length } both in
 HPTK6 / IgG² Fusion } PSV15.IP.CC

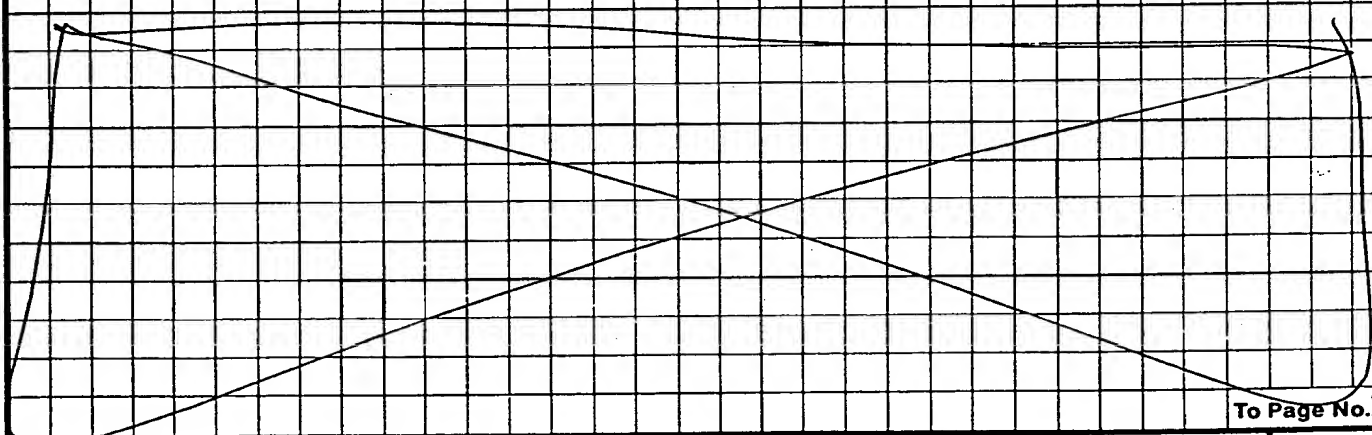
Major cell death is occurring
 ~ 90% of cells are dead.

Micro-colonies are starting to appear → each
 containing ~ 10-20 cells as of now.

Changed media on all plates with
 10ml fresh 37°C pre-warmed

FL2 + 10mM HEPES
 DMEM + 5% d FBS

Continued incubation 37°C O/N w/ 5% CO₂.



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Witnessed & Understood by me, _____

Date _____

Invented by _____

Date _____

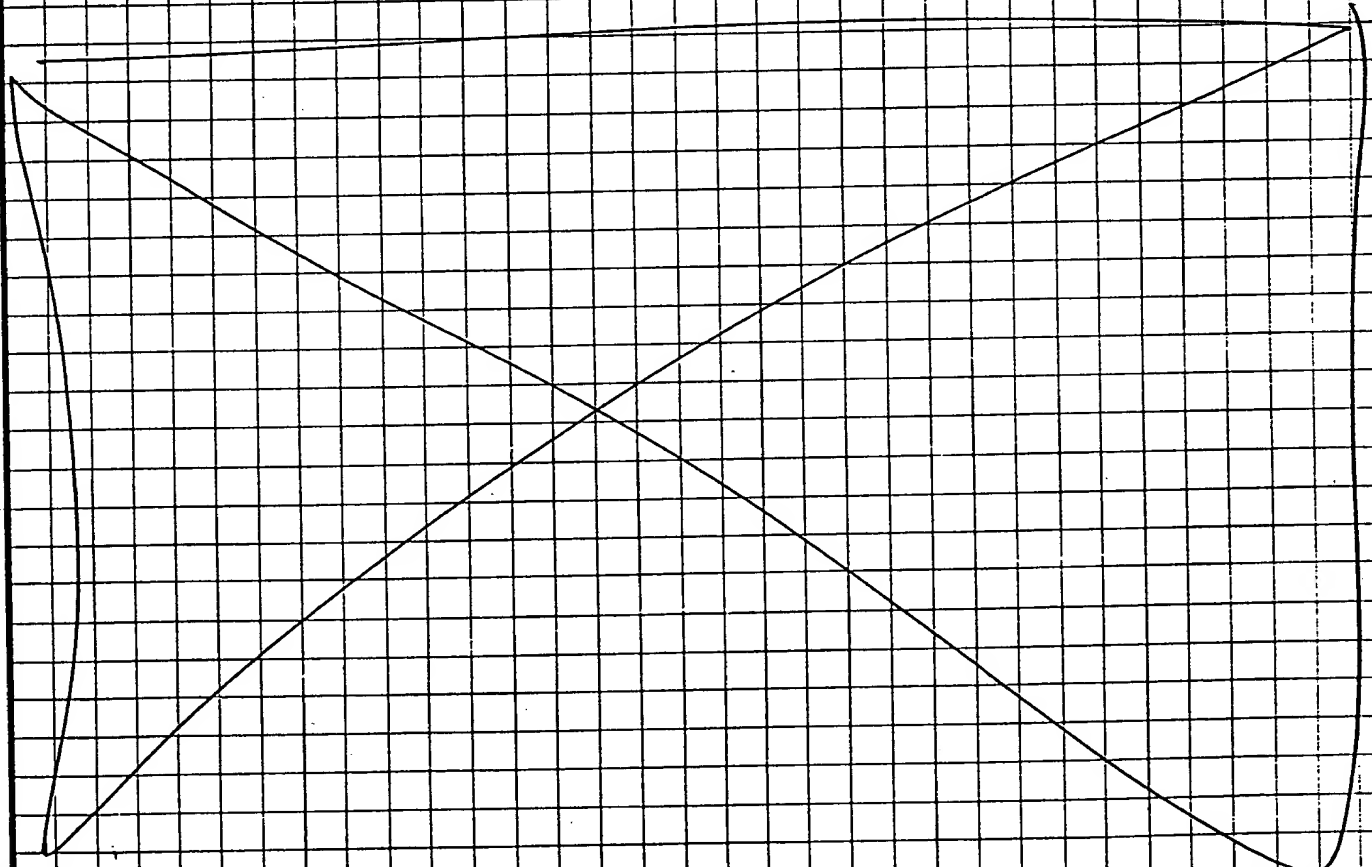
Recorded by W. A. Bacon

TITLE _____

From Page No. 94

Checked cHa cell transfections.
Cell death is continuing
Small colonies are still apparent
(I would guesstimate ~20/plate
and maybe a few more).

Cont Inc 37°C 5% CO₂



To Page No. 96

Witnessed & Understood by me, _____

Date _____

Invented by _____

Recorded by _____

W. H. Bacon

Date _____

Project No. 1713

Exhibit F, pg. 10 of 13

Book No. 17522 TITLE

From Page No. 95

changed media on HPTK6 + HPTK6/FUS
transfection plates

Replaced each w/ 10ml F12 DMEM + 10mM HEPES
5% dFBS

Cont @ 37°C, 5% CO₂ until 5/24/93

To Page No. 1

Witnessed & Understood by me,

Date

Invented by

Recorded by

Date

Book
#

18002

TITLE

Project No
Book No

Exhibit F, pg. 11 of 13

Page

From Page No. 96 Book # 1752.2

Checked CHO cells (HPTK6/IgG2 FUS + HPTK6 F.
cells look fine → colonies are looking good
but are still too small to be seen
by naked eye. Colonies may be
pickable by next week.

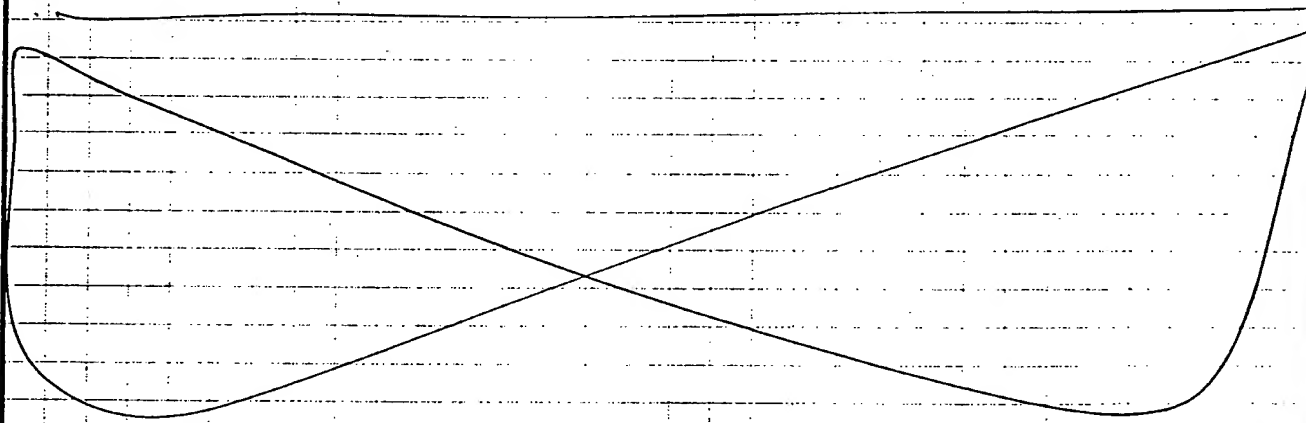
Charged media on all plates

Put 10ml fresh onto each one.

Media is

F12: DMEM
5% d FBS
10mM HEPES.

Cont. Inc @ 37°C w/ 5% CO₂.



Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

W. A. Brown

To Page

From Page No. 7

Checked CHO cell transfection plates.

No contamination

Cells look healthy

Colonies are increasing in size.

Plate is being "seeded" from larger colonies.

Cont. Inc @ 37°C w/ 5% CO₂.

To Page No. 3

Witnessed & Understood by me,

Date

Invented by

Recorded by

Date

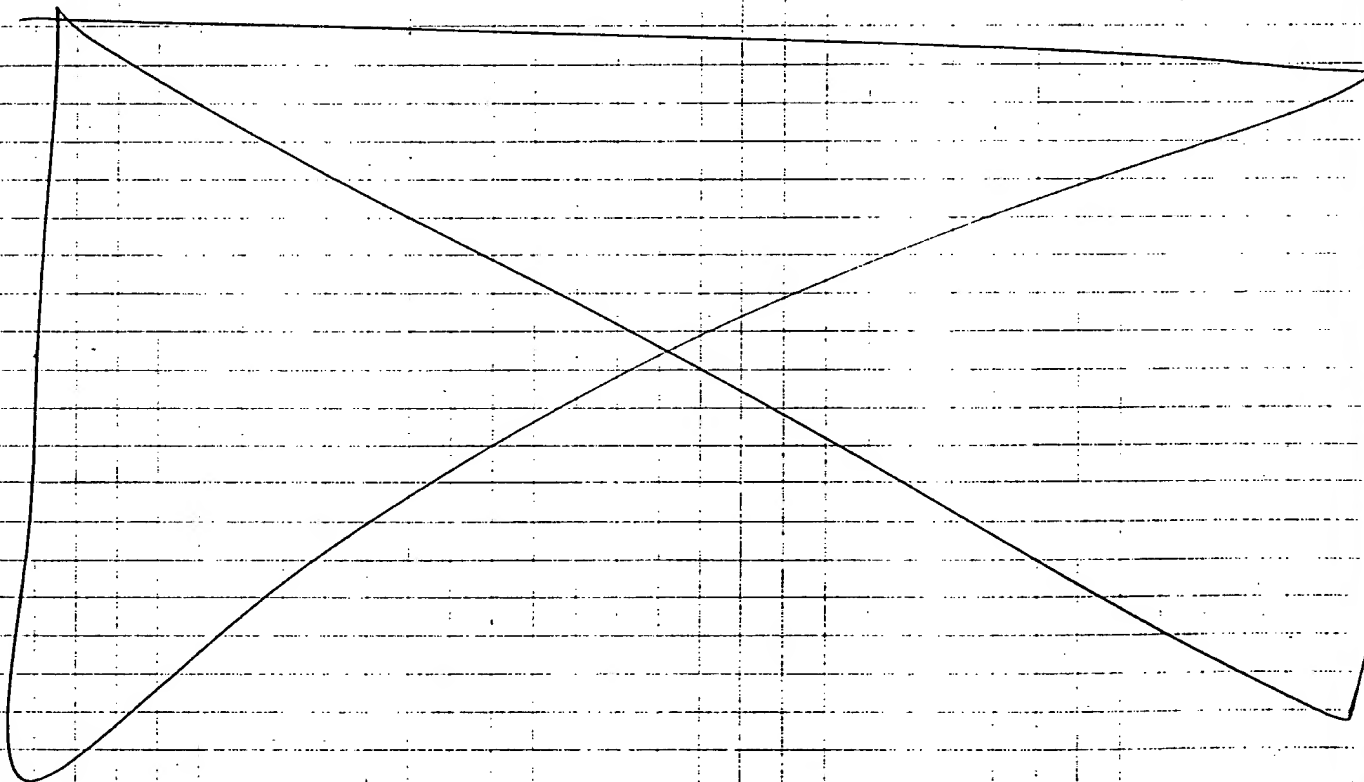
TITLE _____

From Page No. 2

Checked cells → still looking fine
Colonies are now visible to naked eye
but are still way too small to pick.
Changed media → put 10ml fresh on each plate

F12: DMEM
5% of FBS
10 mM HEPES

Cont Inc @ 37°C 5% CO₂



To Page No. 5

Witnessed & Understood by me, _____

Date _____

Invented by _____

Date _____

Recorded by _____

Will Baron

[Signature]